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Modification of Biological Surface Activity of Particles

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The hemolytic activity of fibrous asbestos varieties and of fibrous or granular silica dust can be markedly reduced by adsorption of polymers. Polyanions exert a specific action on asbestos, particularly chrysotile, whereas silica is inactivated by nonionic polymers. A high degree of reduction of the lytic action by comparatively small amounts of the antagonistic polymers can be demonstrated after short exposure to concentrations of 0.1–0.4 mg/ml of appropriate polymers. Inactivation is based on stable adsorption. Repeated washings of inactivated mineral sediments or exposure to elevated temperatures (80–120°C) produced no essential loss of the reduction of lytic potency. In one example, inactivation of chrysotile by sodium alginate, depolymerization by ascorbic acid was also ineffective.

Introduction

The counterparts of polyvinyl-2-pyridine *N*-oxide, (PVPNO) the best known antagonist of hemolytic, cytotoxic, and fibrogenetic properties of silica *(1)* are anionic polymers which prevent the hemolysis of mammalian red cells by asbestos fibers particularly by the most active variety, chrysotile. It is known through the work of Harington et al. *(2–4)* that the lytic property of chrysotile can be inhibited by serum proteins, phosphate ions, and the chelating action of ethylenediaminetetraacetic acid (EDTA). Schnitzer and Pundsack *(5)* first showed the marked antagonism of polyanions in chrysotile hemolysis and pointed out the specificity of this reaction, which is generally limited to asbestos. All polyanions tested so far have shown the antagonistic activity; the list of effective antihemolytic compounds *(6)* comprises synthetic and semisynthetic agents e.g., polystyrene sulfate, the pyran copolymer NSC 46015, carboxymethyl cellulose ether sodium salt (CMC), polysaccharides, such as sodium alginate (SA), vegetable gums (e.g., tragacanth) and mucopolysaccharides, e.g., chondroitin sulfate (CS) and heparin.

Specificity

The specific action on chrysotile hemolysis by polyanions is very marked; it cannot be demonstrated in silica lysis *(6)*. Deviations from strict specificity occur; CS can exert a certain degree of inhibition of silica at high concentrations in the same way as PVPNO under conditions of increased concentration *(7)* or prolonged exposure *(3)* may antagonize chrysotile. Anthophyllite responds to both chrysotile and silica antagonists, and the same observation has been made in tests with sepiolite (Bunescu, unpublished data). Structural characteristics of the fibers are responsible for these reactions. However, as a rule, the phenomenon of specificity is so unequivocal that it is possible to differentiate chrysotile or silica hemolysis by the response to inactivating polymers *(7)*.

These observations are based on experiments in which the hemolytic agents, e.g., asbestos fibers or silica fibers or granular particles and the antagonists were — at least initially — present in their free form in the hemolytic system. Probably adsorption of the antagonists occurred during incubation time. Recent studies have

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been carried out with adsorbed inactivators. The presence or absence of lysis was determined in systems in which all free potential inhibitors had been removed by thorough washing. The specificity principle is also valid under these conditions.

Adsorption of Polymers on Fibers and Granular Particles

It is known *(3)* that PVPNO is bound to silica particles but not to the red cell surface. The same applies to the adsorption of polyanions on asbestos fibers. Exposure of 40–100 mg chrysotile or amphibole fibers to polyanions causes the formation of a fiber–polymer complex which no longer possesses hemolytic properties. The technique of these experiments consists in the adsorption of 10 ml of the polymers in aqueous solution on the fibers at 37°C in a water bath shaker. After removal of the liquid phase of the system, the fiber sediment is washed repeatedly (3–6 times) with 40 ml saline, resuspended in barbiturate buffer of pH 7.3–7.4, and its hemolytic action titrated against a 2% suspension of triply washed red cells from defibrinated sheep blood.

Comparatively small amounts of antihemolytic polymers are sufficient to inactivate larger amounts of chrysotile, as is shown in the example (Table 1) of sodium alginate adsorbed on chrysotile B. The 50% endpoint of the ratio inactivator/fiber is 1–46 to 1–50, independent of the quantity of fibers which corresponds to 200 to 500 times the 50% hemolytic concentration.

Table 1. Effect of adsorption of sodium alginate (SA) on chrysotile B fiber hemolysis.

Chryso B, mg [a]	SA, mg [b]	Ratio chryso B/SA	HC 50, mg/ml [c]	Ratio (chryso B/SA)
100	4.0	1/25	> 12.5	
100	2.0	1/50	> 12.5	1/46
100	1.0	1/100	— [d]	
100	0.5	1/200	—	
40	4.0	1/10	> 5.0	
40	2.0	1/20	> 5.0	
40	1.0	1/40	> 5.0	1/50
40	0.5	1/80	—	
40	0.2	1/200	0.9	
40	0.05	1/800	0.4	
40	0		0.28±0.08	

[a] Total amount of chrysotile exposed to alginate.
[b] Total amount of alginate to which fibers were exposed.
[c] 50% hemolytic concentration.
[d] Not titrated; more than 50% hemolysis.

Inactivation of chrysotile B and amphibole asbestiform fibers by adsorption of a series of anionic polymers is shown in Table 2. The data of this table indicate that marked inactivation can by achieved by the adsorption of the polyanions; polyvinylpyrrolidone (PVP), a silica antagonist, is inactive. As far as the amphiboles are concerned, their low lytic potency has to be considered. Whereas the 50% hemolytic concentration (HC50) of chrysotile B is 0.2 mg/ml ± 0.04, the average lysis by 12.5 mg/ml of amosite (UICC) was 50% (min. 19.5%, max. 85%); the same concentration of crocidolite (UICC) caused an average HC50 of 47.6%, (min. 27.5%, max. 75%), that of anthophyllite (UICC) 80% (min. 45%, max. 95%). Crocidolite was somewhat less sensitive than the other asbestos varieties. Similar results were obtained, if PVP was adsorbed on fibrous quartz or microfine precipitated silica Quso G30 and Quso G32 (Philadelphia Quartz Company), both of particle size 14 μm.

Time Factor of Adsorption

The adsorption process is rapid and as a rule completed almost immediately. In experiments in which 50 mg chrysotile B was exposed to graded amounts of CS or CMC for different time intervals inactivation was obtained at the zero interval which corresponds to approximately 10 min contact of fiber and antagonist, including the time for removal of the solution of the inactivating polymer by centrifugation (Table 3). The effect of the adsorption on the fiber is dependent on the amount of the inactivator, as shown by the lack of activity of CMC 0.5 mg.

Inactivation of the silica particles Quso G30 and G32 by adsorption of PVP was generally a slower process and required higher concentrations of the antagonist with a ratio inactivator/particles of 1/5 to 1/6 for the 10 min interval (Table 4).

Stability of Adsorption

Rapid inactivation of chrysotile fiber hemolysis is accompanied by very stable adsorp-

Table 2. Average of inactivation by polymer adsorption of hemolysis caused by asbestos fibers.

Antagonist [a]	Range, mg	Average % inactivation of lysis			
		Chryso B	Amosite	Crocidolite	Anthophyllite
SA	2.5–50	80±4.4	81±5.4 [b]	75±5.2	62±15.4
CMC	2.5–50	81±3.1	81±3.5	70±9.5	91± 1.3 [c]
CS	2.5–50	85±1.6	90±4.5	72±6.2	82± 9.2 [d]
NSC 46015	2.5–50	85±5.3	79±4.4	72±6.0	65.0 [e]
RS 781	2.5–50	69±4.2	43±2.4	25±7.0	0
PVP	2.5–50	0	29±3.6	0	82± 5.0

[a] SA, sodium alginate; CMC, carboxymethyl cellulose; CS, chondroitin sulfate; NSC 46015, pyran copolymer; RS 781, polystyrene sulfate (high molecular weight).
[b] 100 mg SA included.
[c] 1.0 mg CMC or NSC 46015 included.
[d] 5.0 mg CS and less omitted: <50%.
[e] 2.5–25 mg omitted: <50%.

Table 3. Time factor of adsorption of chondroitin sulfate (CS) and carboxymethyl cellulose (CMC) on chrysotile B. [a]

Inactivator		Inactivation of hemolysis ± SE, %				
Compound	Wt, mg	0 [b]	1 hr	2 hr	4 hr	6 hr
CS	1.0	75±6.4	76±10.7	71±12.7	71±12.7	–
	2.0 [c]	80	82	85	83	–
	4.0	81±5.6	87±4.2	83± 2.8	80± 5.7	–
CMC	0.5 [c]	40	–	–	10	10
	1.0	92±5.0	–	–	90± 4.2	91±4.6
	2.0	92±0	–	–	93± 4.2	92±2.8
	4.0 [c]	92	–	–	90	93
	8.0	86±8.5	–	–	91±1.4	93±0.7

[a] Chrysotile B (UICC), constant amount (50 mg).
[b] Maximal time of contact 10 min.
[c] One determination only.

Table 4. Time factor of adsorption of polyvinylpyrrolidone (PVP) on silica Quso G30 and Quso G32.

Silica		PVP,	Inactivation of hemolysis ± SE, % [a]			
Type	Wt, mg	mg	0 [b]	4	6 hr	Mean
Quso G30	50	1.0	40±20.2	5.0	10.0	24±23.9
		2.0	41±30.0	52±12.9	56±31.7	50± 7.8
		4.0	38±19.7	94± 2.9	94± 1.0	75±32.9
		6.0 [c]	78	97	96	90±10.7
		8.0	86± 6.0	96± 1.0	96± 1.2	89± 7.0
Quso G32	20	1.0	0	0	0	0
		2.0	45±36.7	88± 7.9	96± 1.0	76±26.7
		4.0	86± 8.4	93± 5.5	95± 2.2	91± 5.7
		8.0	93± 4.2	95± 2.8	93± 4.2	94± 1.0

[a] SE = standard error.
[b] Maximal time of contact 10 min.
[c] One determination only.

tion. It has been not possible so far to remove the adsorbed polymer from the fibers.

Storage

Dr. Leineweber of Johns-Manville had prepared an adsorbate of the hemolytic chrysotile Jeffrey which had been treated with SA (25% based on fiber weight). The material, which was submitted as a heavy paste (approximately 70–80% water content), was dried at 37°C, and the resulting white film ground to a coarse powder consisting of fibers. The dried fibers were not hemolytic at a concentration of > 2.5 mg/ml, while the HC50 of untreated fibers was 0.18 mg/ml. When retested after more than 2 yr, dried fibers still failed to show hemolysis at a concentration of 2.5 mg/ml, though they were stored in the form of the wet paste at room temperature.

Washing

Three to six washings with 40 ml saline of chrysotile B fibers (40–100 mg) following the adsorption of SA, CMC, CS, and other polyanions did not change the inactivation of the fibers. Quantitative evaluation of the washed sediments showed that approximately 70–80% of the inactivating amounts of polymers was bound to the fibers. The balance could be estimated by titrating the residual antihemolytic action of the supernatants after adsorption. No activity was found as a rule in the washwaters. A comparable stable adsorption was demonstrated in the adsorption of PVP on fibrous silica; the adsorption by the microfine powders of precipitated silica was also marked.

Elevated Temperatures

The polymers adsorbed on fibers or granular particles are water-soluble compounds; though it was not possible to elute by washing sufficient amounts of the adsorbed antagonists to reactivate the hemolytic activity of the minerals, it appeared possible to remove adsorbed polymers from the mineral surfaces at elevated temperatures. This was, however, not the case. It is evident from the data in Tables 5 and 6 that the inactivation of chrysotile B by SA or CMC and the inactivation of silica G32 by PVP could not be eliminated by heating aqueous suspensions of the adsorbates to 100–125°C for 1 hr.

Depolymerization

These experiments were limited to the depolymerization of sodium alginate by ascorbic acid. Herb et al. *(8)* had shown that ascorbic acid 3 *mM* in phosphate buffer solution depolymerizes SA as measured by determination of reduced viscosity or increased fluidity. These observations have been confirmed by I. Gonda (unpublished data). Attempts to depolymerize SA adsorbed on chrysotile fibers were unsuccessful. Even comparatively high concentrations of ascorbic acid failed to inactivate adsorbed SA. (Table 7). The effect of enzymic breakdown has still to be investigated.

Demonstration of Adsorbed Polymers on the Fiber Surface

If the sediments of chrysotile fibers after adsorption of the mucopolysaccharide CS and the usual thorough washing were treated with the mucopolysaccharide reagent Alcian Blue,

Table 5. Effect of heating on the stability of sodium alginate (SA) and carboxymethyl cellulose (CMC) bound to chrysotile B.

Chrysotile, mg	Bound antagonist		Temp of heating (1 hr), °C	Hemolysis, %	Inactivation, %
	Compound	Wt, mg			
40	SA	5.0	37	9.5	90.5
40	SA	5.0	80±2	12.0	88.0
40	SA	5.0	37	8.0	92.0
40	SA	5.0	125	10.0	90.0
40	CMC	2.0	37	8.0	92.0
40	CMC	2.0	80±2	9.5	90.5
40	CMC	2.0	37	9.0	91.0
40	CMC	2.0	125	7.5	92.5

Table 6. Effect of heating on the stability of polyvinylpyrrolidone (PVP) bound to microfine silica G32.

G32, mg	Bound PVP, mg	Temp of heating (1 hr), °C	Hemolysis, %	Inactivation, %
20	5.0	37	5.5; 5.0	94.5; 95.0
20	5.0	100	5.0; 4.0	95.0; 96.0
20	–	37	100; 100	0; 0
20	–	100	100; 100	0; 0
40	5.0	37	3.5	96.5
40	5.0	100	7.0	93.0
40	–	37	100	0
40	–	100	100	0

Table 7. Effect of ascorbic acid on fiber-bound Na alginate.

Chrysotile, mg	Alginate, mg	Ratio chrysotile/ alginate	Ascorbic acid, mg	Ratio ascorbic acid /alginate	Result
5	2	2.5	10	5	
10	2	5.0	10	5	Antagonistic effect of alginate not affected; no lysis[a]
15	2	7.5	10	5	
25	10	2.5	20	2	
40	40	1.0	40	1	
50	10	5.0	20	2	

[a] No lysis means that the degree of hemolysis was negligible and corresponded to the red cell control (<5%).

significantly more of the dye stuff was adsorbed than by untreated fibers (Table 8).

Comments

Although the experiments described in the preceding section indicate that stable adsorption of inactivators of fiber hemolysis is feasible, the fact that the results are at present limited to hemolysis tests makes it difficult to evaluate the significance of the observations for the prevention of asbestos mediated damage in either other cell systems or *in vivo*. It may be permissible to assume that in analogy to PVPNO in silica *(1)* and the findings by Koshi and Sakabe *(9)* and Koshi et al. *(10)* in serum protein binding, a parallel exists of antihemolytic, anticytotoxic activity *in vitro* and antitoxic action *in vivo*, but this question still requires experimental proof for the polymers. Consideration has also to be given to the toxic properties of the inactivating polyanions. Their stable binding to the fibers may not only inhibit pathogenic effects of the minerals, but also any toxic effect of the comparatively small amounts of the inhibitors. That is not the case if polyanions should be used systemically for prophylactic or therapeutic purposes. A general toxicological property of the polymers is their slow elimination and their storage in cells which can lead to parenchymal injury. Moreover, many of the polyanions exert specific activities which can be favorable, e.g., antiviral action and/or interferon stimulation, anti-inflammatory, and antineoplastic effect *(11)* and inhibition of lung granulomas *(12)*. Other properties may be less favorable, e.g., the anticoagulant effect (heparin, dextran sulfate, polyanethol sulfonate, Liquoid). Extensive toxicological studies will be necessary which have to include the question of carcinogenesis, markedly present in PVPNO *(13, 14)*.

Table 8. Alcian blue (AB) staining of chondroitin sulfate (CS) after adsorption on chrysotile B.

Chrysotile B mg	CS, mg	AB, mg	S [a]	OD [b] W1	OD [b] W2	OD [b] W3	Adsorption, %
50	10	10	8.4±5.0	0.8±0.26	0.7±0.38	0.5±0.11	66±20
50	—	10	14.0±4.1	2.4±0.66	0.9±0.51	0.6±0.10	9±6.7

[a] S = Supernatant after adsorption of AB.
[b] Optical density (filter 580); averages ±SE. W1, W2, W3 = Washwaters 1–3.

Conclusion

Stable adsorption of polyanions on asbestos fibers produces an irreversible inactivation of their hemolytic property. Comparable effects can be obtained by adsorption of nonionic polymers on silica particles. These observations require additional studies in other cell systems and toxicological investigations in order to initiate animal experimentation on prevention of fibrogenesis and carcinogenesis.

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